Video legends

Video 1: Patients with Allan-Herndon-Dudley syndrome present symptoms of parkinsonism in childhood. **(Videos are available from the authors upon request)**

Video 2: Clinical improvement under levodopa/carbidopa therapy. **(Videos are available from the authors upon request)**

Supplementary methods

Supplementary methods 1: *Infantile Parkinsonism-Dystonia Rating Scale (IPDRS)*.

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The IPDRS will soon be submitted to *Movements Disorders* and includes the following subscales:

1. **Non-motor symptoms** (caregiver report)
   - Autonomic dysfunction — 5 items

     Thermoregulation, Respiratory, Gastrointestinal, Sleep, Other
   - Mood dysfunction / emotional lability — 1 item
2. **Motor symptoms** (physical examination, caregiver report for OGC)
   - Oculogyric crises (OGC) (severity, duration, frequency) — 3 items
   - Bradykinesia — 5 items

     Spontaneous movements (global, facial, lower limbs, upper limbs)

     Voluntary movements (upper limbs)
   - Tremor (distribution, severity) — 2 items
   - Rigidity (distribution, severity) — 2 items
   - Dystonia (severity) — 4 items

     Facial, axial, upper limbs, lower limbs
   - Axial hypotonia — 2 items
   - Motor developmental delay — 1 item
3. **Dyskinesia** (physical examination)
   - Hyperkinetic involuntary movements (severity, duration, distribution) — 3 items

     (excludes dystonia, tremor, tics, stereotypies)

Supplementary methods 2: Neuroimaging, volumetry, and PET-studies

Neuroimaging data were collected retrospectively. For **volumetric neuroimaging**, magnetic resonance imaging (MRI) was performed on a 3 Tesla MRI system (Skyra Magnetom, Siemens Healthineers, Erlangen, Germany) with a 64-element head coil. The sagittal T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence took 5 min, 21 s (voxel size 0.9 × 0.9 × 0.9 mm^3, field of view 240 mm^2, repetition time [TR] = 2,300 ms, echo time [TE] = 2.32 ms, TI = 900 ms, flip angle 1 = 8°). Digital imaging and communications in medicine (DICOM) data were converted to the Neuroimaging Informatics Technology Initiative (NIfTI) format using the dcm2niix tool [1]. The resulting NIfTI files were segmented according to the Desikan-Killiany atlas [2] using the Freesurfer v6.0 [3] recon-all pipeline. The resulting segmentation masks were compared with those of healthy subjects [8]. Total white matter brain volume was calculated as follows: total white matter = supra tentorial volume excluding ventricles - (total gray matter + left cerebellar cortex + right cerebellar cortex)/3.

Positron emission tomography (PET) list-mode data were acquired in a Biograph mMR PET/MRI scanner (Siemens Healthineers, Erlangen, Germany) over a 60 minute period, initiated concurrently with intravenous bolus administration of 86 MBq (14-year-old patient) and 49 MBq (5-year-old patient) 18F-DOPA. List-mode data were reconstructed with standard parameters (OSEM, 3 iterations, 21 subsets, correction for attenuation and scatter) and in 20 frames (3 x 20 s, 3 x 1 min, 3 x 2 min, 3 x 3 min, 7 x 5 min, 1 x 6 min). PET was corrected for head motion between frames and the individual PET mean images and individual T1 images were co-registered. Each T1 image was spatially normalized using the unified segmentation approach with default setting and the computed normalization parameters were then applied to the co-registered PET frames and the individual subcortical brain segmentation masks from FSL. PET data were analyzed using Statistical Parametric Mapping 12 (Welcome Department of Imaging Neuroscience, Institute of Neurology, London, http://www.fil.ion.ucl.ac.uk/spm/) and based on a previously described pipeline [4]. Dopamine synthesis capacity was quantified as 18F-DOPA rate constant per min (K_i), which was estimated voxel by voxel using Gjedde-Patlak linear graphical analysis [5]. Radioactivity time curves in the cerebellum mask from the automated anatomical labeling atlas [6] were used as input function. The linear fit was restricted to the time interval 5-60 min after injection. Mean K_i values were extracted from the voxelwise map for each individual subcortical regions-of-interest (caudate nucleus, putamen, accumbens nucleus, pallidum, amygdala, hippocampus, thalamus). Normal values of n=44 adult 18F-DOPA rate constants (Ki) in regions of interest were digitized from published figures [7].

References

1. Li X, Morgan PS, Ashburner J, Smith J, Rorden C. The first step for neuroimaging data analysis: DICOM to NIfTI conversion. J. Neurosci. Methods 2016;264:47–56.
2. Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage 2006;31:968–80.
3. Fischl B. FreeSurfer. NeuroImage 2012;62:774–81.
4. Deserno L, Huys QJM, Boehme R, Buchert R, Heinze HJ, Grace AA, et al. Ventral striatal dopamine reflects behavioral and neural signatures of model-based control during sequential decision making. Proc. Natl. Acad. Sci. U. S. A. 2015;112:1595–600.
5. Patlak CS, Blasberg RG. Graphical Evaluation of Blood-to-Brain Transfer Constants from

Multiple-Time Uptake Data. Generalizations. J. Cereb. Blood Flow Metab. 1985;5:584–90.

6. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. NeuroImage 2002;15:273–89.

7. Lorenz RC, Gleich T, Buchert R, Schlagenhauf F, Kühn S, Gallinat J. Interactions between glutamate, dopamine, and the neuronal signature of response inhibition in the human striatum. Hum. Brain Mapp. 2015;36:4031–40.

8. Rutherford S, Fraza C, Dinga R, Kia SM, Wolfers T, Zabihi M, et al. Charting brain growth and aging at high spatial precision. eLife 2022;11:e72904.

Supplementary methods 3: Differentiation of iPSC to dopaminergic neurons, microscopic imaging, and measurement of intracellular biogenic amines

Differentiation of iPSCs to dopaminergic neurons. To generate dopaminergic neurons from iPSCs, we obtained the NCRM-1 iPSC line (https://hpscreg.eu/cell-line/CRMi003-A) commercially from RUCDR Infinite Biologics (RUCDR). Cells were cultured on Geltrex (Cat# A1413302, Gibco) coated six-well plates (Cat# 353046, Falcon) in mTESR1 (Cat# 85851, Stem Cell Technologies) under hypoxic conditions (5% CO_2, 5% O_2, 37°C). Cell culture medium was changed daily and cells were enzymatically dissociated with dispase (Cat# 354235, Corning).

Differentiation of iPSCs to neuronal progenitor cells (NPCs) was performed following published protocols [1,2]. Briefly, iPSC colonies were detached with dispase. Cell clusters were transferred into a uncoated non-tissue culture six-well plate (Cat# 351146, Falcon) and cultivated for two days in **medium 1** [Knockout DMEM (Cat# 10829018, Gibco), 20% knockout serum replacement (Cat# 10828-028. Gibco), 1 mM beta-Mercaptoethanol (Cat# 21985-023, Gibco), 1% MEM non-essential amino acids (Cat# 11140035, Invitrogen), 1% Pen/Strep, 1% Glutamine (Cat# 25030-024, Invitrogen), 10 µM SB-431542 (Cat# 130-106-543, Miltenyi Biotech), 1 µM Dorsomorphin (Cat# P5499-5MG, Merck), 3 µM CHIR 99021 (Cat# 4423, Trocis), and 0.5 µM Purmorphamine]. When cells had formed spherical clusters, medium was changed to **medium 2** [1:2 DMEM F-12 (Cat# 31330-038, Gibco), 1:2 Neurobasal (Cat# 21103-049, Gibco), 1:200 N2 (Cat# 17502-048, Gibco), 1:100 B27 (without vitamin A, Cat# 12587010, Gibco), 1% Pen/Strep, 1% Glutamine, 10 µM SB-431542, 1 µM Dorsomorphin, 3 µM CHIR 99021, and 0.5 µM Purmorphamine]. After another two days incubation, medium was changed to **medium 3** [1:2 DMEM F-12, 1:2 Neurobasal, 1:200 N2, 1:100 B27 (without vitamin A), 1x MycoZap Plus-CL (Cat# VZA-2012, Lonza), 2 mM Glutamine, 3 µM CHIR 99021, 0.5 µM Purmorphamine, and 150 µM ascorbic acid (Cat# A7631, Sigma Aldrich)]. After another two days, cell spheres were shredded and transferred into a Geltrex-coated 24-well plate (cell culture-treated). For the first day, 1 µM ROCK inhibitor (Cat# 1254, Trocis) was added to the cell culture medium. NPC were cultivated Geltrex-coated six-well cell culture plates (Cat# 353046, Falcon) in medium 3 under normoxic conditions (5% CO_2, 37°C). Medium was changed every other day. NPCs were split once a week with Accutase (Cat# A1110501, Gibco).

The differentiation of NPCs to dopaminergic neurons was performed as described before [1]. Briefly, NPCs were expanded in a 6-well plate and cultivated in **medium 3** under normoxic conditions. After seven days, cell culture medium was changed to **medium 4** [1:2 DMEM F-12, 1:2 Neurobasal, 1:200 N2, 1:100 B27 (with vitamin A, Cat# 17504044, Gibco), 1x MycoZap Plus-CL, 2 mM Glutamine, 0.5 µM Purmorphamine, 150 µM ascorbic acid, and 100 ng/mL FGF8a (Cat# 100-25A, Pepro-tech)] (day 1). After two more bidaily medium changes, cells were expanded in more wells of a Geltrex-coated 6-well plate and on Geltrex-coated glass coverslips in culture **medium 5** [1:2 DMEM F-12, 1:2 Neurobasal, 1:200 N2, 1:100 B27 (with vitamin A, Cat# 17504044, Gibco), 1x MycoZap Plus-CL, 2 mM Glutamine, 150 µM ascorbic acid, 10 ng/ml BDNF (Cat# 450-02, Pepro-Tech), 10 ng/ml GDNF (Cat# 450-10, Pepro-Tech), 1 ng/mL TGF-b3 (Cat# 100-36E, Pepro-Tech), and 500 µM dbcAMP (Cat# D0260, Sigma Aldrich), supplemented with 0.5 µM Purmorphamine] (day 8). After 48 hours, the medium was changed

to **medium 5 without Purmorphamine supplementation** (day 10) and cells were cultivated in this medium for the rest of the experiment with bidaily medium change.

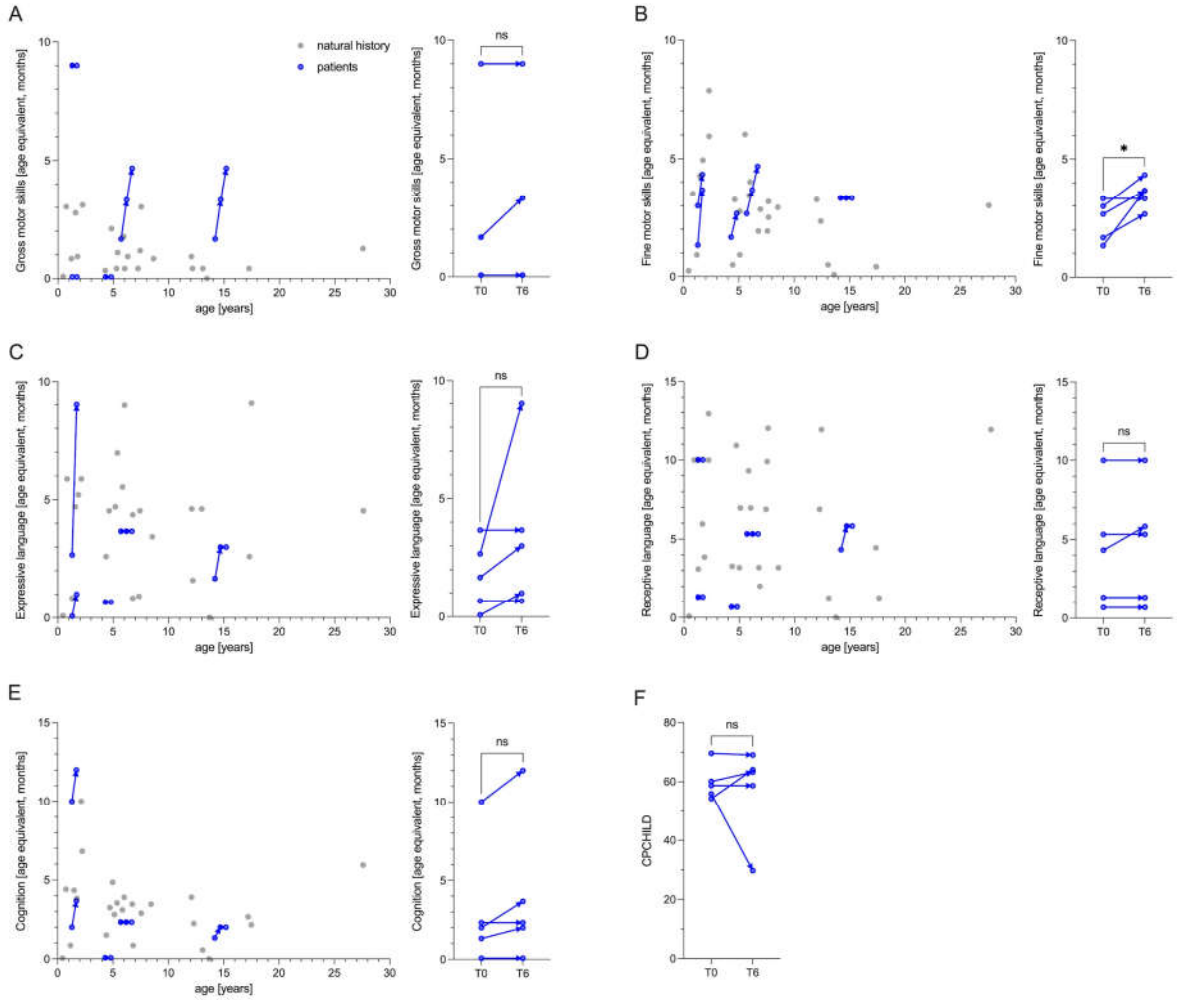
Immunofluorescence labeling and imaging. For immunofluorescence labeling, after fixation, samples were thoroughly rinsed with PBS, blocked in PBS, containing 5% normal donkey serum (Cat# ab7475, Abcam) and 0.3% Triton X-100 (Cat# T-9284, SIGMA), and incubated with a mixture of well-established primary antibodies (rabbit-anti-MCT8: 1:200, Cat# NBP2-57308, Novus Biologicals; mouse-anti-TUJ1: 1:1000, Cat# T8578, Sigma-Aldrich; chicken-anti-TH: 1:200, Cat# ab76442, Abcam) diluted in 0.5% normal serum and 0.03% Triton X-100 at 4° C overnight. After several washing steps with PBS, samples were incubated with corresponding secondary antibodies (donkey-anti-rabbit-Cy3: 1:250, Cat# AP182C, Merck; donkey-anti-mouse-A488: 1:250, Cat# ab150105, Abcam; donkey-anti-chicken-647: 1:250, Cat# A78952, Thermo Fisher Scientific) for one hour at room temperature. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI, 1:1000, Cat# D1306, Invitrogen) in PBS, and samples were mounted in fluorescence mounting medium (Mowiol 4-88, Carl Roth) followed by coverage with cover slips. For immunofluorescence labeling, neuronal cultures were fixed with 100% ethanol for 10 seconds and stained as described [3]. A THUNDER Imager DMi8 with a Leica DFC9000 GT camera and LAS(X) software (Leica Application Suite (X), version 3.7.4.23463, Leica Microsystems) was used for image acquisition.

Measurement of intracellular biogenic amines. For the measurement of intracellular biogenic amines in dopaminergic neurons, we used neuronal cultures differentiated for 22, 35, and 42 days (n=2 cultures from each time point). 28 h and 4 h before cell extraction, we added 150 µM of the precursor levodopa (Cat# 3788, Tocris) to the media. For extraction, we added ice-cold methanol to the neuronal cultures, scraped the cells from the dish and transferred them to a solvent-resistant Eppendorf tube for incubation for 10 min at -20°C. After centrifugation at 24,000 x *g*, 4°C for 5 minutes, the supernatant was stored in liquid nitrogen and subsequently analyzed for biogenic amines, 5-MTHF, and pterins. The measurement of intracellular biogenic amines was done as published [4].

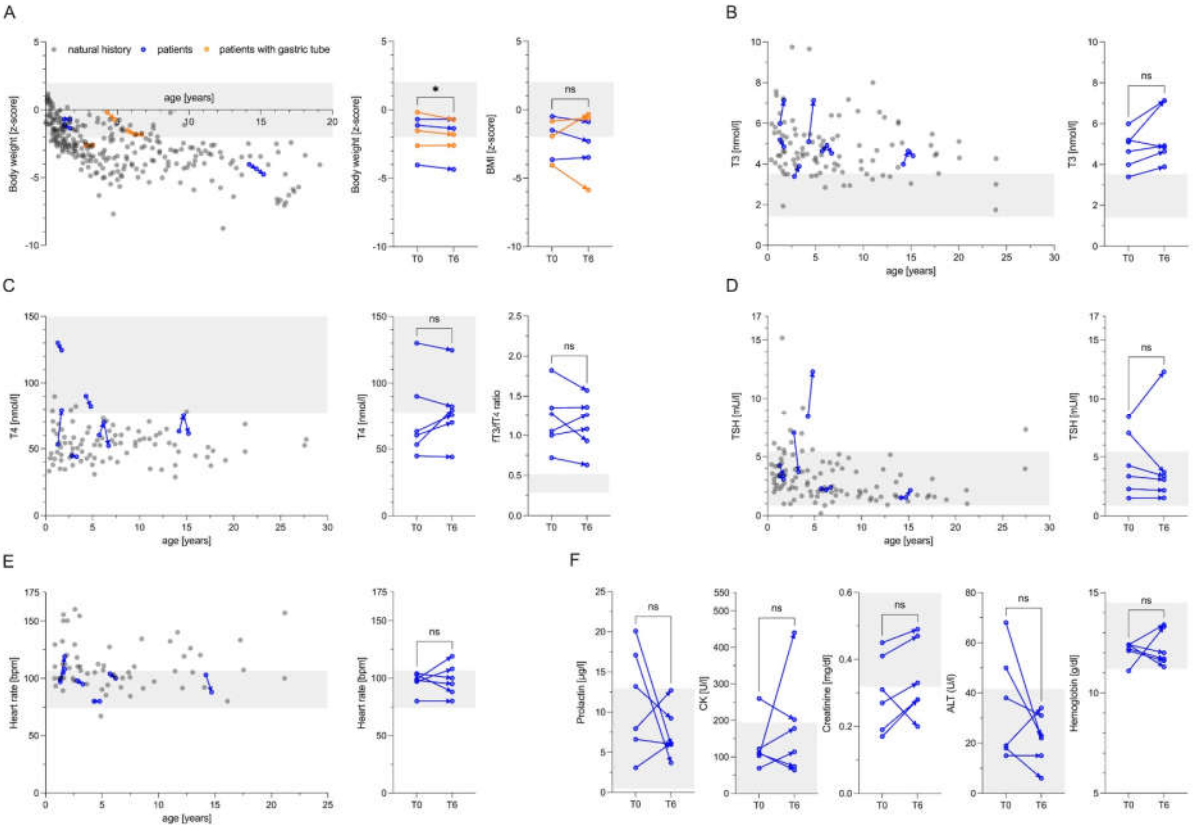
References

1. Reinhardt P, Glatza M, Hemmer K, Tsytsyura Y, Thiel CS, Höing S, et al. Derivation and expansion using only small molecules of human neural progenitors for neurodegenerative disease modeling. PloS One 2013;8:e59252.
2. Zink A, Lisowski P, Prigione A. Generation of Human iPSC-derived Neural Progenitor Cells (NPCs) as Drug Discovery Model for Neurological and Mitochondrial Disorders. Bio-Protoc. 2021;11:e3939.
3. Wilpert NM, Krueger M, Opitz R, Sebinger D, Paisdzior S, Mages B, et al. Spatiotemporal Changes of Cerebral Monocarboxylate Transporter 8 Expression. Thyroid 2020;30:1366–83.
4. Mahajani S, Raina A, Fokken C, Kügler S, Bähr M. Homogenous generation of dopaminergic neurons from multiple hiPSC lines by transient expression of transcription factors. Cell Death Dis. 2019;10:898.

Supplementary figures



Supplementary figure 1: Development and quality of life under levodopa/carbidopa treatment. (A-E) Patients exhibited significant improvement in fine motor skills when assessed using the *Bayley Scales of Infant and Toddler Development Third Edition (BSID-III)*, while no notable changes were observed in other categories. The natural history data were extracted with ImageJ from the published work referenced in the text [11]. The hypothesis of a normal distribution was always tested using the Shapiro-Wilk test ($\alpha = 0.05$). In the event that the data were normally distributed, the statistical significance of the differences between the groups was evaluated through the application of a paired t-test. In the event that the data were not normally distributed, the Wilcoxon test was applied. ns, not significant; *, $p \leq 0.05$. **(F)** No significant improvement in the quality of life, as measured by the *Cerebral Palsy Child Health Index of Life with Disabilities (CPCHILD)*, could be reported. One family experienced a period during which a gastric tube was placed in their child and hip surgery was performed, and thus reported a reduced quality of life. The data were not normally distributed according to the Shapiro-Wilk test. The statistical significance of differences between the groups was tested by applying the Wilcoxon test. ns, not significant.



Supplementary figure 2: No adverse drug reactions were seen under levodopa/carbidopa treatment. Adverse drug reactions of the levodopa/carbidopa treatment were neither reported by parents **(A-F)** nor could any significant negative changes in patients' (depicted in blue) body mass index (BMI), heart rate, or laboratory tests be identified. Three patients were fitted with a gastric tube (depicted in orange). The natural history data (depicted in gray) were extracted from published work using the image processing software ImageJ. The hypothesis of a normal distribution was tested using the Shapiro-Wilk test ($\alpha = 0.05$). In the event that the data exhibited a normal distribution, the statistical significance of the differences between the groups was evaluated through the application of a paired t-test. Conversely, in instances where the data did not display a normal distribution, the Wilcoxon test was employed. ns, not significant; *, $p \leq 0.05$; fT3, free triiodothyronine; fT4, free thyroxine; TSH, thyroid stimulating hormone; bpm, beats per minute; CK, creatine kinase (as a marker for muscle fiber damage); ALT, alanine transaminase (as a marker for liver function damage).